International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 1163-1170
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Qualitative and Quantitative Analysis of *Z. jujuba* Leaf, Fruit and Seed Extracts

Ponmani. S[1]., Selvankumar, T[2]., Kala. K[3] and Gopinath L. R[4]

[1]Department of Biotechnology, Padmavani Arts and Science College for Women, Opp. Periyar University, Salem – 636 011.

[2]Department of Biotechnology, Kalippatti (Po), Tiruchengode (Tk), Namakkal (Dt), Tamil Nadu, India. 637 501.

[3]Former Regional Joint Director, Collegiate of Education, Tirchy.

[4]Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women (A), Namakkal, Tamilnadu, India.

Received: 12 Oct 2018 / Accepted: 11 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: lrgopinathnamakkal@gmail.com

Abstract

Zizyphus jujuba belongs to *Rhamnaceae* family which comprises about fifty genera and more than 900species. It is well known for its biological properties and there may be unknown phytochemical compounds present in the plant. Present work was studied on the qualitative and quantitative analysis of *Z. jujuba* leaf, fruit and seed using different extracts. Many compounds show presence in methanol extract and the quantitative analysis shows high in phenolic compounds.

Keywords

Phytochemical analysis, compounds

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INTRODUCTION

Medicinal plants are a pool of biologically active compounds with therapeutic properties has been well-known, and in view of the fact that ancient time they have been used worldwide for the treatment of various ailments, like asthma, skin disorders, gastro-intestinal problems, urinary and respiratory complications, hepatic and cardiovascular disease (Cousins and Huffman, 2002; Tian *et al.,* 2014). The interest on insist for more plant derived drugs, are considered as safe while compared to synthetic drugs, is increasing fast. The medicinal value of these plants deception in chemical substances that produce an encouraging physiological action on the human body and because plants synthesize tremendously assorted range of chemical compounds, they represent an immense potential for the development and discovery of new pharmaceuticals (Chesney *et al.*, 2007).

The phytochemical compounds or secondary metabolites are well known for its biological properties such as antioxidant activity, antimicrobial activity, anticancer activity, detoxification enzymes, immune system stimulation, diminish of platelet aggregation and modulation of hormone metabolism There are more over thousand known and many unknown metabolites in plant system. It is clearly known that plants produce these phytochemicals to protect themselves, however recently scientists reveal that many phytochemicals can also protect human against many diseases (Narasinga Rao, 2003).

Zizyphus jujuba Mill, known as Red date, Chinese date and Bera belongs to Rhamnaceae family which comprises about fifty genera and more than 900 species. The *Ziziphus Jujuba* is a native to Asia, Southern Europe and is very common in some Middle Eastern countries (Sun *et al.,* 2011). The broadly cultivated jujubes are *Z. mauritiana* or *Z. jujuba* which is also known as the Indian jujuba or ber whereas the wild variety of *Zizyphus* is *Z. nummularia*. The *Z. jujuba* have been cultivated over enormous area of the world (Mukhtar *et al*, 2004). *Z. jujuba* is a deciduous tree which grows very well in Mediterranean climate and can bear heat and drought. *Z. jujuba* tree bears fruits that are edible and delicious with various shapes and sizes. These fruits exhibit great nutritional and medicinal value but have not been subjugated commercially on the proper scale.

Different parts of *Z. jujuba* possess numerous medicinal properties (Golmohammad *et al.*, 2013). The reasons for medicinal property of this plant are because of a few substances like riboflavin, ascorbic acid, bio flavanoids, thiamine and pectin-A. *Z. jujuba* fruits are extremely rich in vitamins such as ascorbic acid (vitamin C), thiamine (vitamin B1) and riboflavin (vitamin B2). One fruit of *Z. jujuba* per day would complete the diet necessities for Vitamin C and Vitamin B for an adult man when compared to other edible fruits (Farooq, 2003).

MATERIALS AND METHODS

Collection and Identification of the Sample

Z. jujuba leaf, fruit and seeds was collected from the Namakkal district. They were washed and dried under shade in room temperature, then grinded and stored. The powdered samples were dissolved in solvents chloroform, methanol, ethanol, petroleum ether, acetone and water. The extract was filtered using Soxhlet apparatus.

Qualitative analysis of secondary metabolites

Test for alkaloids

Dargendorff's test:

Few drops of dargendorff's reagent was added to 1-2 ml of the plant extract and observed for the formation of the orange red precipitate.

Mayer's test:

Few drops of mayer's reagent was added to 2ml of the plant extract and observed for the formation of brown precipitate.

Wagner's test:

Few drops of wagner's reagent was added to the 2 ml of the plant extract and observed for the formation of the brown precipitate.

Test for flavanoids

Alkaline test:

Few drops of alkaline solution were added to 1 ml of the plant extract and observed for the formation of yellow color.

Pews test

5 mL of the aqueous solution of the water extract was mixed with 0.1 g of metallic zinc and 8ml of concentrated sulphuric acid. The mixture was observed for red colour as indicative of flavonols.

Shinoda's test:

Few drops of shinoda's solution was added to 3ml of the plant extract and observed for the formation of the pink or red color.

Tests for Lignins

Labat test

When gallic acid is added to the test sample, it results in the formation of olive green colour.

Lignin test

When furfuraldehyde is added to the test sample a red colour appears indicating the presence of lignin.

Tests for Tannins

Ferric chloride test

When few drops of ferric chloride were added to sample solution a blackish precipitate appears.

Gelatin test

When gelatin and water were added to test samples formation of white precipitate was resulted.

Tests for Phenolic Compounds

Ferric chloride test

When few drops of ferric chloride were added to sample solution a blackish precipitate appears.

Ellagic acid test

When 5% glacial acetic acid and 5% sodium nitrite were added to test samples a muddy niger brown colour appears, which is a positive result for phenols.

Phenols test

The extract (500mg) was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green clour indicated the presence of phenolic compounds.

Test for Terpenoids

Liebermann Burchard's test

Few drops of Liebermann Burchard's reagent was added to 2ml of the plant extract and observed for the formation of reddish-brown ring.

Tests for Sterols

Libermann-Buchard test

When samples were treated with few drops of acetic anhydride, boiled and few drops of concentrated

sulphuric acid from the sides of the test tube were added, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of steroids.

Salkowski test:

Few drops of concentrated sulphuric acid were added to the test samples in chloroform, a red colour appears at the lower layer indicates the presence of sterols.

Tests for Glycosides

Legal's test

When the test samples were treated with pyridine and sodium nitroprusside solution blood red colour appears.

Kellar Kiliani test

1ml of concentrated sulphuric acid was taken in a test tube then 5ml of extract and 2ml of glacial acetic acid with one drop of ferric chloride were added, reaction shows formation of a blue colour.

Concentrated Sulphuric acid test

Conc.H_2SO_4 was added to test sample which resulted in appearance of reddish colour.

Molisch test

When alpha naphthol and concentrated H2SO4 were added to test samples reddish violet ring at junction of two layers was resulted.

Test for saponin

Lead acetate test

Few drops of lead acetate solution were added to the 2ml of the plant extract and observed for the formation white precipitate.

Foam formation test

Few gram (0.5mg) of leaf powder was dissolved with 20ml of distilled water in a measuring jar and continuously shake for 15 minutes and observed for the formation of foam up to 1-2cm.

Test for protein

Millon's test

Few drops of millon's reagent were added to the 2ml of the extract and observed for the formation of red color.

Biuret test

Few drops of biuret solution were added to 2ml of the extract and observed for the appearance of violet color.

Test for carbohydrate

Molisch's test

Few drops of molisch's reagent was added to the 2ml of the plant extract and observed for the formation of reddish violet ring.

Quantitative analysis of Secondary Metabolites

Quantitative analysis of Secondary Metabolites was done by using the standard procedure prescribed by Alkaloids by Harborne *et al*., (1973), Flavanoids by Kumaran, *et al*., (2006), Saponins by Obadani *et al*., (2001), Terpenoids, tannins by (Makkar, 2003), phenolics by Donald., *et al*., 2001).

RESULTS

The phytochemical analysis of the leaf, fruits and seeds of *Z. jujuba* different solvents extracts were tabulated (Table 1, 2 and 3).

Quantitative analysis of the leaf, fruit and seed methanol extract showed high content of alkaloids in leaf, flavonoids in seeds, tannins in seeds, phenolic compounds, terpenoids and saponins in fruits and steroids in seeds (Figure 1). Ash content was high in leaves, fat in seeds, fibre in leaves, proteins in seeds, carbohydrates in fruits and other contents high in leaves.

Table 1. Phytochemical test of *Ziziphus jujuba* leaf extracted with different solvents.

S. No.	Phytochemical test		Solvents					
			Ethyl Acetate	Methanol	Ethanol	Petroleum Ether	Hexane	Water
I	**Alkaloids**							
1	Alkaloids	Dragendroff	-	+	+	-	+	-
		Iodine Test	-	+	+	-	+	-
		Wagners Test	-	+	+	-	+	-
II	**Phenolic compounds**							
2	Flavonoids	Alkaline	+	+	+	-	+	-
		Pews Test	+	+	+	-	+	-
		Shinoda Test	+	+	+	-	+	-
3	Lignin	Lignin	-	+	+	-	+	+
		Labat Test	-	+	+	-	+	+
4	Tannins	Ferric chloride	-	+	+	+	+	+
		Gelatin Test	-	+	+	-	+	+
5	Phenols	Ferric chloride	+	-	-	-	+	+
		Ellagic Test	-	+	+	+	+	-
		Phenol Test	-	-	+	+	+	-
III	**Terpinoids**							
6	Terpinoids	Libermann burchard	+	+	+	+	+	-
7	Sterols	Libermann- Burchard Test	-	-	-	-	-	-
		Salkowski Test	+	+-	+	-	+	+
8	Glycosides	Legals	-	+	+	+	+	+
		Keller Killani Test	-	+	+	+	+	-
		Glycosides Test	-	-	+	+	+	+
		Conc. H_2SO_4	-	+	+	+	+	+
		Molisch's Test	-	-	+	-	-	+
9	Saponins	Lead acetate	-	+	+	-	+	+
		Foam Test	-	+	+	-	+	+
		Haemolysis Test	-	+	+	-	+	+
IV	**Proteins**							
10	Protein	Millons	-	+	+	+	+	+
V	**CARBOHYDRATES**							
11	Carbohydrate	Molichs	+	+	+	+	+	+

Table1.2 Phytochemical test of *Ziziphus jujuba* fruit extracted with different solvents.

S. No.	Phytochemical test		Solvents					
			Chloroform	Methanol	Ethanol	Petroleum Ether	Acetone	Water
I	**Alkaloids**							
1	Alkaloids	Dragendroff	-	+	+	+	+	+
		Iodine Test	-	+	+	-	+	-
		Wagners Test	+	+	+	-	+	-
II	**Phenolic compounds**							
2	Flavonoids	Alkaline	+	+	+	+	+	+
		Pews Test	+	+	+	-	+	-
		Shinoda Test	+	+	+	-	+	-
3	Lignin	Lignin	-	+	+	-	+	+
		Labat Test	-	+	+	-	+	+
4	Tannins	Ferric chloride	-	+	+	+	+	+
		Gelatin Test	-	+	+	-	+	+
5	Phenols	Ferric chloride	+	-	-	-	+	+
		Ellagic Test	-	+	+	+	+	-
		Phenol Test	-	-	+	+	+	-
III	**Terpinoids**							
6	Terpinoids	Libermann burchard	+	+	+	+	+	-
7	Sterols	Libermann- Burchard Test	-	-	-	-	-	-
		Salkowski Test	+	+-	+	+	+	+
8	Glycosides	Legals	-	+	+	+	+	+
		Keller Killani Test	-	+	+	+	+	-
		Glycosides Test	-	-	+	+	+	+
		Conc. H2SO4	-	+	+	+	+	+
		Molisch's Test	-	-	+	-	-	+
9	Saponins	Lead acetate	+	+	+	+	+	+
		Foam Test	-	+	+	-	+	+
		Haemolysis Test	-	+	+	-	+	+
IV	**Proteins**							
10	Protein	Millons	-	+	+	+	+	+
V	**CARBOHYDRATES**							
11	Carbohydrate	Molichs	+	+	+	+	+	+

Table 1.3 Phytochemical test of *Ziziphus jujuba* seed extracted with different solvents.

S. No.	Phytochemical test		Solvents					
			Chloroform	Methanol	Ethanol	Petroleum Ether	Acetone	Water
I	**Alkaloids**							
1	Alkaloids	Dragendroff	-	+	+	+	+	+
		Iodine Test	-	+	+	-	+	-
		Wagners Test	-	+	+	-	+	-
II	**Phenolic compounds**							
2	Flavonoids	Alkaline	+	+	+	-	+	+
		Pews Test	+	+	+	-	+	-
		Shinoda Test	+	+	+	-	+	-
3	Lignin	Lignin	-	+	+	-	+	+
		Labat Test	-	+	+	-	+	+
4	Tannins	Ferric chloride	-	+	+	+	+	+
		Gelatin Test	-	+	+	-	+	+
5	Phenols	Ferric chloride	+	-	-	-	+	+
		Ellagic Test	-	+	+	+	+	-
		Phenol Test	-	-	+	+	+	-
III	**Terpinoids**							
6	Terpinoids	Libermann burchard	+	+	+	+	+	-
7	Sterols	Libermann- Burchard Test	-	-	-	-	-	-
		Salkowski Test	+	+-	+	-	+	+
8	Glycosides	Legals	-	+	+	+	+	+
		Keller Killani Test	-	+	+	+	+	-
		Glycosides Test	-	-	+	+	+	+
		Conc. H_2SO_4	-	+	+	+	+	+
		Molisch's Test	-	-	+	-	-	+
9	Saponins	Lead acetate	+	+	+	+	+	+
		Foam Test	-	+	+	-	+	+
		Haemolysis Test	-	+	+	-	+	+
IV	**Proteins**							
10	Protein	Millon's	-	+	+	+	+	+
V	**CARBOHYDRATES**							
11	Carbohydrate	Molich's	+	+	+	+	+	+

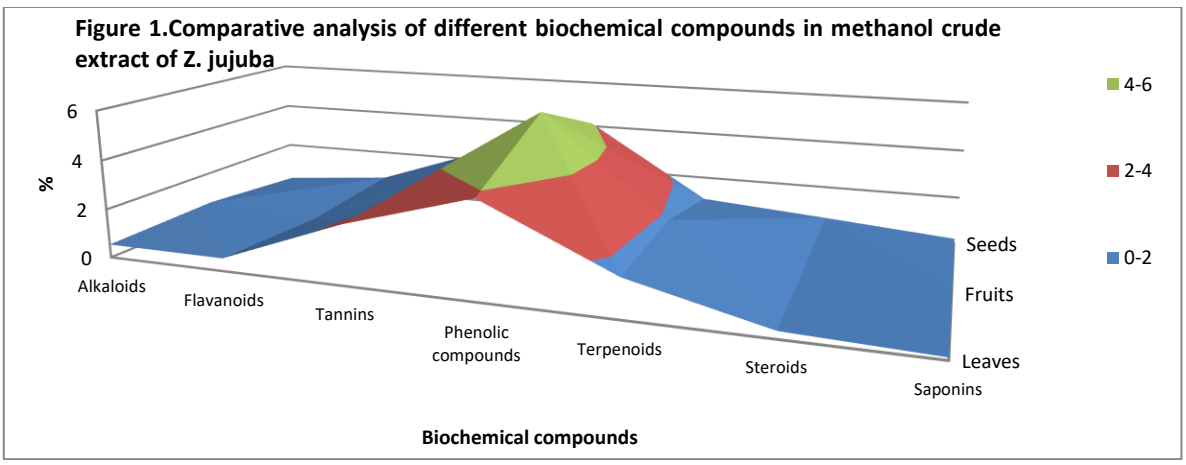
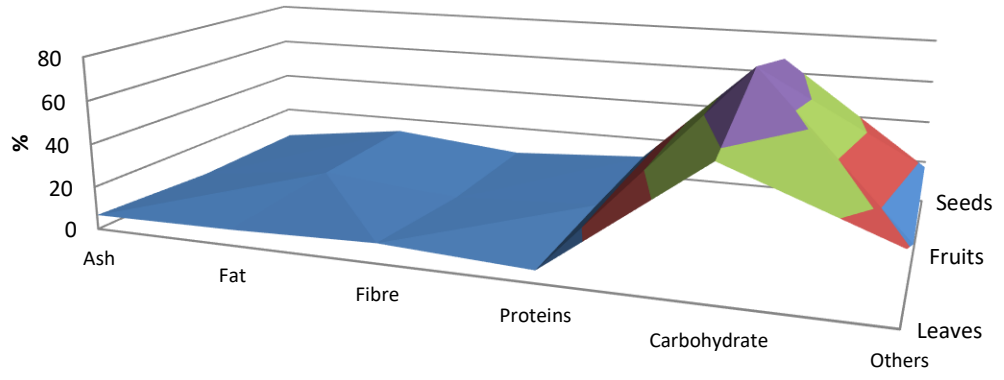


Figure 2. Comparative analysis of different nutrients in methanol crude extract of Z. jujuba



Nutrients

DISCUSSION

Plants are accomplished with a range of phytochemical compounds like alkaloids, phenolic acids, vitamins, tannins, terpenoids, lignins, stilbenes, flavanoids, quinones, coumarins, amines, betalains and additional metabolites which were rich in antioxidant activity (Zheng *et al.,* 2001). Many studies have revealed that, these antioxidant substances possess anti-bacterial, anti-tumor, anti-mutagenic, anti-inflammatory, anti antherosclerotic, anti-carcinogenic, anti-fungal and anti-viral activities (Giner *et al.,* 2002; Miller *et al.,* 1995). The intake of natural antioxidant has been coupled with reduced risk of diabetes, cancer, cardio vascular diseases and other diseases linked with delaying ageing.

Plant products have been part of phyto medicines since time immemorial. Phyto chemicals can be derived from any part of the plant such as flowers, bark, leaves and seeds etc to which any part of the plant may contain dynamic components (Cragg and David, 2001). Knowledge of the chemical constituents of plants is pleasing because such information will be of worth for the synthesis of complex chemical substances. Phytochemical screening of various medicinal plants is reported by many researchers (Siddiqui *et al.*, 2009; Ashok kumar *et al.*, 2010; Chitravadivu *et al.*, 2009).

One such medicinal plant was *Z. jujuba* and this plant contains lot of phyto chemical compounds. In the present study the phyto chemical from this plant was qualitatively analyzed using different extracts. But in the methanol extract the secondary metabolic compounds vigorously existed. The similar work also done by Essam *et al*, in ethanolic extract of *Z. jujuba* stem bark.

CONCLUSION

The study concludes that methanol extract was effective for extracting the secondary metabolites from *Z. jujuba*. A phenolic compound was high in compared to other compounds in leaf, fruits and seeds of methanol extract. The carbohydrate content was high in leaf, fruits and seeds of methanol extract in compared to other contents.

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